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Comparative Acute and Subchronic Toxicity of Ethylene Glycol Monopropyl Ether and Ethylene Glycol Monopropyl Ether Acetate

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The acute toxicity of ethylene glycol monopropyl ether (EGPE) and ethylene glycol monopropyl ether acetate (EGPEA) was determined in a series of standardized tests. The oral LD_{50} in rats was 3089 and 9456 mg/kg EGPE and EGPEA, respectively. Skin irritation was slight following an occluded single dose application of either compound to the guinea pig abdomen. The dermal LD_{50} for guinea pigs was 1 to 5 mL/kg and greater than 20 mL/kg EGPE and EGPEA, respectively. EGPE produced a very weak positive sensitization response in one of five guinea pigs. No positive response was elicited when 10 guinea pigs were similarly challenged with EGPEA. EGPE produced transient moderate to severe eye irritation in rabbits while EGPEA produced slight eye irritation. Subchronic toxicity was determined in a series of oral and inhalation studies. Groups of 10 male rats were dosed with 15, 7.5, 3.75 or 1.88 mmole/kg EGPE and 30, 15 or 7.5 mmole/kg EGPEA by gavage 5 days/week for 6 weeks. Hemoglobinuria was seen at least once at all dose levels of both compounds. EGPE had little effect on feed consumption or body weight gain, while body weight gain was reduced in the two high dose groups exposed to EGPEA and feed consumption was reduced at all dose levels. Hematologic changes were seen at all dose levels of both compounds. Absolute and/or relative spleen weights were increased at all but the lowest EGPE dose level and at all EGPEA dose levels. Gross and histopathologic examinations revealed significant effects on the spleen of animals exposed to EGPE and on the spleen, liver, kidney and testes of animals exposed to EGPEA. The no-observed effect level (NOEL) for splenic changes was 1.88 mmole/kg EGPE. A NOEL for hematology was not established. The NOEL for liver and testicular changes were 15 and 7.5 mmole/kg EGPEA, respectively while a NOEL for hematologic, splenic and renal changes was not established. Groups of 10 rats (5M, 5F) were exposed to 800, 400, 200 or 100 ppm EGPE or EGPEA 6 hr/day, 5 days/week for a total of 11 exposures. Body weight gains in all exposure groups were comparable to controls. Hemoglobinuria was seen only after the first or second exposure in males and females exposed to 800 ppm EGPE and in males exposed to 400 ppm EGPE. Males and females exposed to 800 ppm EGPEA and females exposed to 400 and 200 ppm EGPEA also exhibited hemoglobinuria. Hematologic changes were seen in males and females exposed to 800 and 400 ppm EGPE and EGPEA. Absolute and/or relative spleen weights were increased at 800 and 400 ppm EGPE and EGPEA. Gross and histopathologic examinations revealed splenic effects in males and females exposed to 800 and 400 ppm EGPE and 800 ppm EGPEA. The NOEL was 200 ppm EGPE and 100 ppm EGPEA.

Introduction

The acute and subchronic toxicity of ethylene glycol monomethyl ether (EGME), ethylene glycol monomethyl ether acetate (EGMEA), ethylene glycol monoethyl ether (EGEE), and ethylene glycol monoethyl ether acetate (EGEEA) has been studied in several animal species. The acute toxicity of these compounds following single-dose oral and inhalation administration is relatively low. Reported oral LD_{50} values for the rat are: EGME, 2.46 to 3.4 g/kg (*1,2*); EGMEA, 3.39 to 3.93 g/kg (*3,4*); EGEE, 3.46 to 5.5 g/kg (*1,2,5,6*); and EGEEA, 5.1 g/kg (*4*). Reported inhalation LC_{50} values for the rat are: EGME, 2000 ppm/4 hr (*7*); EGMEA, 7000 ppm/4 hr (*3*); EGEE, 4000 ppm/4 hr (*7*); and EGEEA, 1500 ppm/8 hr (*8*). Skin irritation was mild following application of all four compounds to rabbits (*1,9*). All four compounds were mildly irritating to the rabbit eye with conjunctival and corneal irritation being transitory (*1*).

Repeated oral administration of the four compounds produces testicular atrophy and leukopenia, with kidney damage and hematuria reported for EGME, EGEE, and EGMEA (*1*). The principal effects following inhalation exposure to all the compounds are hematologic. Injury to lung, liver and kidneys has also been reported (*1*).

The objective of the following study was to compare and contrast the acute and subchronic toxicity of the next higher homologs in this series of straight chain glycol derivatives, i.e., ethylene glycol monopropyl

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ether (EGPE) and ethylene glycol monopropyl ether acetate (EGPEA).

Methods and Materials

Test Compounds

EGPE and EGPEA were obtained from Tennessee Eastman Company (Kingsport, TN). Physical–chemical properties are compared in Table 1.

The purity of the EGPE and EGPEA was determined by gas chromatography and mass spectroscopy and was found to be about 99.5 and 99.6%, respectively. The main impurities were 2-*n*-butoxyethanol, triethylene glycol *n*-butyl ether, and isopropyl ethanol for the EGPE tested. EGPE, acetic acid, propyl acetate, hexyl acetate, ethylene glycol monobutyl ether acetate and EGEEA (50–100 ppm) were impurities identified in the EGPEA tested.

Single-Dose Oral LD_{50} Studies

Groups of five male rats [COBS/CD/(SD)BR] from Charles River Breeding Laboratories (Wilmington, MA) weighing 150 to 200 g were given oral doses of 10.5, 21.0, 42.0 and 84.0 mmole/kg (EGPE and EGPEA) and 168.0 mmole/kg (EGPE only), roughly equal to 1090, 2180, 4360, 8720 or 17,470 mg/kg EGPE and 1530, 3070, 6140, or 12,280 mg/kg EGPEA. Dose administration was by gavage, undiluted, using a glass syringe fitted with a polypropylene catheter. The animals were individually housed in suspended wire-bottom cages. Water and feed were available *ad libitum*, except the animals that were fasted 16 to 20 hr prior to treatment. General appearance and activity, toxicologic signs and mortality were checked twice daily except weekends and holidays. The appearance of stools and urine on the dropping trays was noted and individual body weights were done prior to dosing and at the end of the 2-week observation period. The LD_{50} and the 95% confidence interval was calculated by the method of Weil (*10*).

Single-Exposure Inhalation LC_{50} Studies

Groups of four male rats [COBS CD (SD)BR] from Charles River Breeding Laboratories (Wilmington, MA) weighing 150 to 200 g were exposed to target concentrations of 2000, 1000, 500, 250 or 0 ppm EGPE and 1000, 500, 250 or 0 ppm EGPEA. Animals received a single 6-hr exposure in 20-L glass bell-jar inhalation chambers. The highest vapor concentration was chosen as the most practical upper limit achievable. Vapors of the test materials were produced by slight heating of the liquid contained in a three-necked round-bottomed flask. The vapors were diluted with oil-free compressed air as necessary prior to entering the bell-jar chambers. Concentrations were determined at least once per hour by infrared analysis (Miran IA-Foxboro/Wilks, Inc., Norwalk, CT). The animals were observed for mortality and clinical signs of toxicity daily and for mortality only on weekends. Body weights were determined twice weekly.

Table 1. Physicochemical properties of EGPE and EGPEA.

	EGPE	EGPEA
Synonym	2-Propoxyethanol	2-Propoxyethyl acetate
Structural formula	$C_3H_7OCH_2CH_2OH$	$C_3H_7OCH_2CH_2OOCCH_3$
Molecular weight	104.15	146.21
Boiling point, °C	149.5	170-1
Melting point, °C	< −90	< −45
Specific gravity	0.91	0.93
Vapor pressure, mm Hg at 25°C	1.3	0.5
Flash point, °F	120	142

Single-Dose Dermal Studies

Primary skin irritation was determined by application of the compound to the depilated abdomen of guinea pigs dosed with 1, 5, 10 or 20 mL/kg EGPE or EGPEA under an occlusive wrap for 24 hr. The compound was placed on a Webril pad which was glued to a strip of rubber dental dam. The strip of dental dam was wrapped around the trunk of the guinea pig. The anterior and posterior edges of the dam were taped to the animal's body. Observations were made immediately following removal of the wrapping (24 hr after dosing), and 1 and 2 weeks after dosing. Body weights were determined before dosing and 2 weeks after dosing. The dermal LD_{50} was estimated as greater than the largest dose causing no mortality or between two doses as appropriate.

Repeated Topical Application Studies

Groups of five guinea pigs received 0.5 mL EGPE or EGPEA applied topically on close-clipped back skin. The test material was applied undiluted. At 24 hr after application, the treated area of the skin was depilated and the skin observed for the presence of abnormalities. On subsequent days, except Sunday, the treated area of the back skin was similarly treated for a total of 10 doses. The skin was clipped again on day 7 and depilated 24 hr after the last (10th) dose. Succeeding observations were compared to those made 24 hr after dosing and exacerbation was considered present if there was an increase in severity in any one sign. Animals were weighed prior to the first dose and after the last dose.

Sensitization Studies

A group of five guinea pigs received a footpad injection (0.05 mL) of Freund's complete adjuvant. After 1 week, 0.3 mL of a 1% solution (acetone-base solvent) of the test material was applied (drop-on) to the depilated backs of the guinea pigs to assess irritation potential. Subsequently, two groups of guinea pigs (five

each for EGPE studies and ten each for EGPEA studies) received a footpad injection (0.05 mL) of Freund's complete adjuvant with and without 1% of the test material. One week later, a challenging dose of 0.3 mL of the appropriate solution (1%) of EGPE or EGPEA was dropped on the depilated back skin. Observations were made 24 and 48 hr later.

Primary Eye Irritation Studies

One eye each of six rabbits was treated by dropping 0.1 mL EGPE or EGPEA into the conjunctival sac formed by pulling the lower eyelid away from the eye. The lids were then held together for approximately 1 sec and released. Immediately after the eyelids were released the conjunctival sac and the surface of the treated eye in three of the rabbits was copiously washed with distilled water. The untreated eye of each rabbit was used as a control. The eyes were observed and evaluated promptly (within 1 min) and 1, 24, 48 hr and 14 days after treatment. Ocular lesions were graded according to guidelines (Draize) recommended by the U. S. Department of Health, Education, and Welfare. Fluorescein sodium ophthalmic solution, U.S.P., was dropped on the cornea of each eye 24 hr after treatment. The eye and conjunctival sac were flushed with distilled water and the presence or absence of staining was noted.

Subchronic Studies

Six-Week Oral Studies. Forty male rats [COBS CD (SD)BR)] from Charles River Breeding Laboratories (Wilmington, MA) weighing 239 ± 13 g were randomly divided into four equal groups. Doses of EGPE, 1560, 780, and 390 mg/kg (15, 7.5, and 3.75 mmole/kg) which were approximately equivalent to 1/2, 1/4, and 1/8 the acute oral LD_{50}, were administered undiluted by gavage five days per week for six weeks. Control animals received a volume of distilled water equal to the largest volume given a treated animal. All doses were recalculated weekly to adjust for body weight. Another 40 male rats weighing 216 ± 6 g were treated similarly with 4386, 2193 and 1097 mg/kg (30, 15, and 7.5 mmole/kg) EGPEA. These doses were also approximately equivalent to 1/2, 1/4 and 1/8 the acute oral LD_{50} of EGPEA. An additional group of 10 rats weighing 232 ± 25 g received EGPE at a lower concentration 195 mg/kg (1.88 mmole/kg, 1/16 the LD_{50}) in an attempt to establish a no-effect level. All animals were housed individually in suspended wire cages and Purina Rodent Chow 5001 and water, via an automatic watering system, were available *ad libitum*.

Two-Week Inhalation Studies. Twenty-five male and twenty-five female rats [COBS CD (SD)BR] from Charles River Breeding Laboratories (Wilmington, MA) weighing 212 ± 7 and 187 ± 5 g, respectively, were randomly divided into five equal groups of each sex. Both sexes were exposed to 800, 400, 200, 100, or 0 ppm EGPE 6 hr/day, 5 days/week for a total of 11 exposures. Controls were treated identically to the test groups except that exposure was to filtered air only. All animals were housed individually in suspended wire cages and Purina Rodent Chow 5001 and water, via an automatic watering system, were available *ad libitum* during nonexposure intervals only.

Vapors were produced by heating (up to 40°C) the EGPE contained in three-necked round-bottomed flasks. The vapors were mixed with metered air and further diluted as necessary prior to entering 4200 L inhalation chambers. Chamber atmospheres were quantitatively analyzed at least once per hour by a gas chromatograph equipped for automated sampling and analysis. Periodic measurements of nongaseous airborne material were made in each chamber using a Royco five-channel particle analyzer to insure the absence of aerosol. Animal cage positions within each chamber were changed daily in a sequential manner. Chamber temperature and humidity were monitored at least once per hour and were maintained at approximately 21°C and 50% relative humidity.

Another 25 male and 25 female rats weighing 212 ± 8 and 183 ± 7 g, respectively, followed a similar regimen but were exposed to 800, 400, 200, 100 and 0 ppm of EGPEA. These animals were exposed in 420 L chambers and analyses of chamber atmospheres was by a Miran IA infrared analyzer equipped for automated sampling and analysis.

Clinical Observations. Individual body weights were recorded at least once weekly in the 6-week oral and 2-week inhalation studies. Feed consumption was recorded with body weights in the oral study. Feed consumption was not determined in the inhalation studies. Animals were observed twice daily for clinical signs of toxicity in the oral studies, and prior, during, and after exposures in the inhalation studies. Animals were observed for mortality on weekends. Animals that died spontaneously were autopsied promptly and moribund animals were euthanized with CO_2 and autopsied. Tissues were collected for histopathologic examination. Organ weights, clinical chemistries and hematology were not done for animals dying spontaneously.

Hematology and Clinical Chemistry. All animals were fasted for 16 to 20 hr prior to autopsy. At termination, the survivors were anesthetized by CO_2 inhalation (oral studies) or by IP injection of sodium pentobarbital (inhalation studies). Blood was drawn from the inferior vena cava just prior to autopsy. Animals were killed by exsanguination. Hematologic analyses (hemoglobin concentration, hematocrit, red and white blood cell counts, platelet counts and red cell indices) were performed using an ELT-8 Laser Hematology Analyzer (Ortho Instruments, Westfield, MA). Differential white cell counts were performed by microscopy. Serum clinical chemistry determinations were done with either a Beckman Enzyme Activity Analyzer System TR (Beckman Instruments Inc., Fullerton, CA) for alanine amino-transferase, aspartate aminotransferase, and alkaline phosphatase or with a

COBAS-BIO Centrifugal Analyzer (Roche Analytical Instruments, Nutley, NJ) for lactic dehydrogenase, urea nitrogen, creatinine, sorbitol dehydrogenase, and glucose.

Gross and Histopathology. The heart, liver, kidneys, testes, ovaries (inhalation study only), brain and spleen were trimmed and weighed for organ/body weight comparisons.

The following tissues were fixed in 10% buffered formalin, embedded in paraffin, sectioned at 5 μm, stained with hematoxylin eosin, and examined by light microscopy: nasal passages, trachea, lungs, thymus, heart, tongue, esophagus, stomach, duodenum, jejunum, ileum, cecum, colon, liver, kidneys, urinary bladder, pituitary gland, adrenal glands, pancreas, thyroid glands, parathyroid glands, spleen, mesenteric lymph nodes, bone marrow, brain, salivary glands, testes, epididymides, accessory sex organs (males), and ovaries, uterus, fallopian tubes and vagina. Eyes were fixed in Zenker's solution.

Analysis of Numerical Data

Body weights, organ weights and, where applicable, all numerical data, were analyzed by using a one-way analysis of variance ($p \leq 0.05$) and Duncan's multiple range test.

Results

Acute Studies

The acute toxicity of both compounds is compared in Table 2. The oral LD_{50} values were 3089 mg/kg EGPE and 9456 mg/kg EGPEA. The 95% confidence intervals were 2090 to 4576 and 7957 to 11,239 mg/kg, respectively. Clinical signs of toxicity were abnormal respiratory patterns, weakness, anorexia, hemoglobinuria, tremors (EGPE animals only), prostration, and death.

The 6-hr inhalation LC_{50} for male rats was greater than 2132 ppm EGPE and greater than 934 ppm EGPEA, the highest practical vapor concentrations achievable without concomitant aerosol exposure. No mortality was observed at any exposure concentration tested. Body weight gains over the 14-day observation period were normal. Clinical signs of toxicity were hemoglobinuria at concentrations of 1121 and 2132 ppm EGPE and 934 ppm EGPEA. No other significant clinical signs of toxicity were observed.

Both compounds produced slight skin irritation when applied to the depilated abdomen of guinea pigs under an occlusive wrap for 24 hr. Erythema and edema observed at 24 hr resolved within 1 week. Only desquamation was seen 1 and 2 weeks after removal of the wrap. All animals exposed to 5 mL/kg EGPE died, while all animals exposed to 1 mL/kg EGPE and 20 mL/kg EGPEA survived. Thus, the dermal LD_{50} was estimated to be between 1 and 5 mL/kg EGPE (about 1 to 5 g/kg) and greater than 20 mL/kg EGPEA (about 20 g/kg). Ten daily applications of EGPE or EGPEA to the clipped backs of guinea pigs over an 11-day period slightly exacerbated the responses seen after the initial application. No mortality was observed.

When tested using a standardized skin procedure, a weak positive response was seen in one out of five guinea pigs challenged with EGPE while no positive responses were elicited in ten guinea pigs challenged with EGPEA. Thus, EGPEA does not appear to be a sensitizer while EGPE, at most, has a very low sensitization potential.

EGPE produced a moderate to strong eye irritation causing severe erythema and moderate edema of the conjunctivae. Iritis, and staining of the adnexa and cornea of rabbit eyes was also apparent. Some degree of corneal opacity was seen in all eyes tested. All responses, however, resolved by 14 days. EGPEA was a slight eye irritant causing erythema and edema of the conjunctivae and nictitating membrane. All responses resolved within 48 hr. Prompt irrigation had a slightly palliative effect with both compounds.

Subchronic Studies

Six-Week Oral Studies. Mortality following administration of EGPE and EGPEA is compared in Table 3. Spontaneous deaths, 2/10, 3/10 and 1/10, dosed with 15, 7.5 and 3.75 mmole/kg EGPE, respectively, were seen immediately after dosing. Two of the three deaths at 7.5 mmole and one at 3.75 mmole/kg appeared to be due to aspiration of the chemical into the lung. No mortality was seen in the groups dosed with 1.88 and 0 mmole/kg EGPE. In contrast, 6/10 rats died after two or three

Table 2. Comparative acute toxicity of EGPE and EGPEA.

Type of dose	Species	EGPE	EGPEA
Oral LD_{50}	Rat	3089 mg/kg	9456 mg/kg
Inhalation LC_{50}	Rat	> 2132 ppm/6hr	> 934 ppm/6hr
Skin			
Dermal LD_{50}	Guinea pig	1–5 mL/kg	> 20 mL/kg
Single-occluded	Guinea pig	Slight	Slight
Repeated-open	Guinea pig	Slight exacerbation	Slight exacerbation
Sensitization	Guinea pig	Weak (1/5)	None (0/10)
Eye	Rabbit	Transient mod.-sev.	Slight

Table 3. Mortality following administration of EGPE and EGPEA: 6-week gavage study.

	30 mmole/kg	15 mmole/kg	7.5 mmole/kg	3.75 mmole/kg	1.88 mmole/kg	0
EGPE (mg/kg)	—[a]	1560	780	390	195	0
Mortality	—	2/10	3/10	1/10	0/10	0/10
EGPEA (mg/kg)	4386	2193	1097	—	—	0
Mortality	10/10	0/10	0/10	—	—	0/10

[a]Study not conducted at these doses.

doses of 30 mmole/kg EGPEA. Additionally, two rats were killed after two doses because of inanition and anorexia and two rats died after 18 doses. No mortality was seen in groups dosed with 15, 7.5, or 0 mmole/kg EGPEA.

Body weight gains of all groups exposed to EGPE were statistically similar to controls except for a slight weight loss in the high dose group on day 3 only (Fig. 1). Feed consumption (Fig. 2) in all groups was similar to controls for the entire study except for a slight decrease during the first 2 weeks in the high dose group (15 mmole/kg EGPE). In contrast, a dose-dependent decrease in body weight gain (Fig. 3) and feed consumption (Fig. 4) was seen in animals exposed to EGPEA. In addition, an initial weight loss during the first week only, was seen in the two surviving animals exposed to 30 mmole/kg EGPEA.

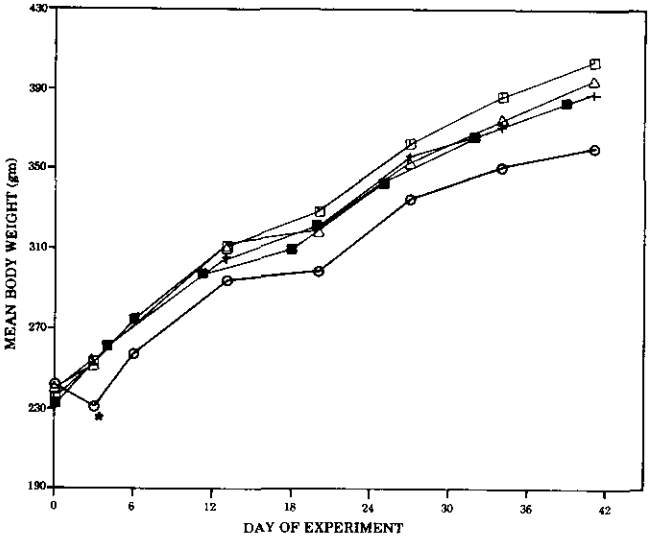

FIGURE 1. Mean body weight of male rats exposed to EGPE (6-week gavage study): (○) 15; (Δ) 7.5; (+) 3.75; (■) 1.88 mmole/kg; (□) controls. An asterisk indicates that values are significantly different from controls ($p \leq 0.05$).

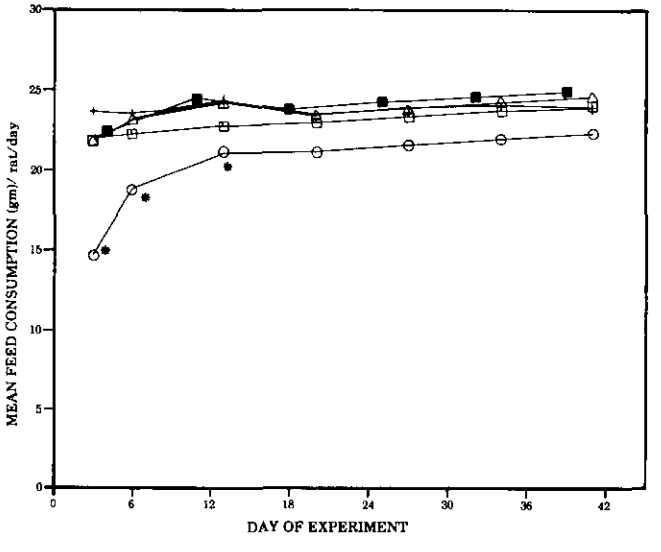

FIGURE 2. Feed consumption of male rats exposed to EGPE (6-week gavage study): (○) 15; (Δ) 7.5; (+) 3.75; (■) 1.88 mmole/kg; (□) controls. An asterisk indicates that values are significantly different from controls ($p \leq 0.05$).

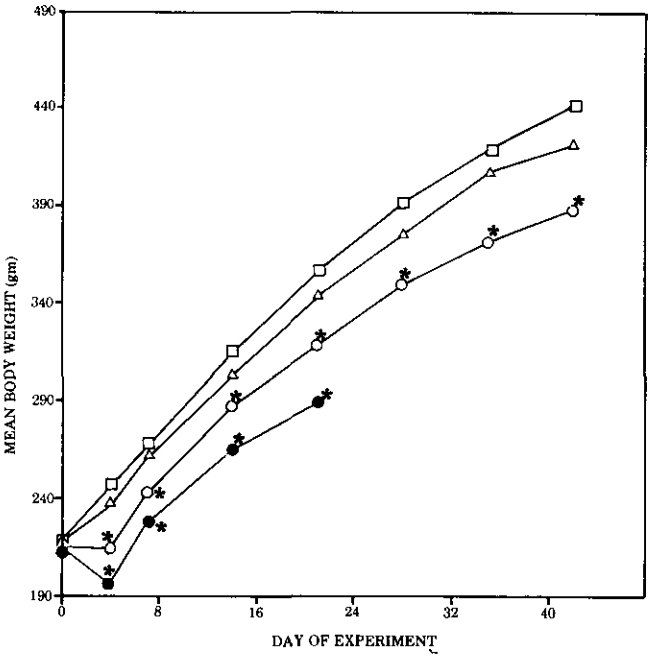

FIGURE 3. Mean body weight of male rats exposed to EGPEA (6-week gavage study): (●) 30; (○) 15; (Δ) 7.5 mmole/kg; (□) controls. An asterisk indicates that values are significantly different from controls ($p \leq 0.05$).

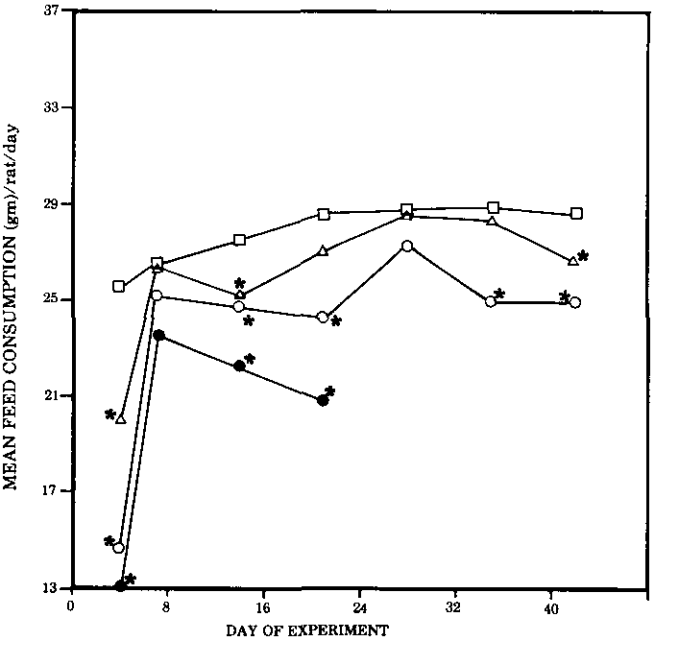

FIGURE 4. Feed consumption of male rats exposed to EGPEA (6-week gavage study): (●) 30; (○) 15; (Δ) 7.5 mmole/kg; (□) controls. An asterisk indicates that values are significantly different from controls ($p \leq 0.05$).

Table 4. Hemoglobinuria following administration of EGPE and EGPEA: 6-week gavage study.[a]

	30 mmole/kg	15 mmole/kg	7.5 mmole/kg	3.75 mmole/kg	1.88 mmole/kg
EGPE	—[b]	10/10	10/10	10/10	2/10
EGPEA	10/10	10/10	10/10	—	—

[a]Observed on dropping trays. All dose groups were affected but number of animals involved and severity of response decreased as study progressed.
[b]Study not conducted at these doses.

Red discolored urine diagnosed as hemoglobinuria (Ames N-Multistix-C reagent strip) was seen on dropping trays in all groups exposed to EGPE and EGPEA (Table 4). The number of animals involved and the frequency of the observation decreased as the study progressed. Other clinical signs seen in some but not all rats administered the high and intermediate doses of EGPE included weakness, labored breathing, prostration, and rales, and at the high dose of EGPEA, narcosis and sialorrhea.

Both compounds produced effects on red blood cells (Fig. 5). Significant dose-dependent decreases in hemoglobin concentration, hematocrit (except the EGPE groups) and red blood cell counts were seen in survivors at all dose levels at termination. Additional blood changes which reflected toxic effects were increases in platelet count, nucleated red blood cells, anisocytosis, macrocytosis and poly- and hypochromasia. White blood cell counts were normal in all groups exposed to EGPE, while a dose-dependent increase in white blood cell counts was observed in animals exposed to 7.5 and 15 mmole/kg EGPEA. The increase was statistically significant in the high dose group only. Differential white blood cell counts were normal at all dose levels of both compounds.

EGPE and EGPEA produced no compound-related effects on clinical chemistries examined. Changes observed were, at most, slight, not dose related and reflected spurious changes frequently observed in this species.

Only the high dose (15 mmole/kg) of EGPE produced a slight, but significant, reduction of terminal body weight while both 15 and 7.5 mmole/kg EGPEA groups had a significantly reduced terminal body weight. A dose dependent increase in absolute and relative spleen weights was observed among all groups exposed to either compound (Fig. 6). The increases in absolute weights were statistically significant at the 15 and 7.5 mmole doses of EGPE and the relative spleen weights were significant at all doses except 1.88 mmole/kg EGPE. A reduction in absolute testicular weight but not in relative weight was seen in animals exposed to 15 mmole/kg EGPEA. The testes weights of the 7.5 mmole/kg EGPEA rats and all groups of EGPE were normal. All other organ weights were normal or reflected decreased body weight gain.

Gross pathologic examination of the 30 mmole/kg EGPEA animals that died or were killed after two or three doses revealed serous atrophy of the bone marrow (4/8), minimal to moderate hemorrhage in the nonglandular portion of the stomach (8/8), and some discoloration of the liver and kidneys (4/8).

Histologic examination of tissues collected from the eight rats that died or were killed after two or three doses of 30 mmole/kg EGPEA revealed minor cytoplasmic vacuolation (3/8) and minor to moderate degeneration of the seminiferous tubules in the testes (4/8). Degenerated sperm cells were seen in the lumen of the epididymides of six of these eight rats. Additional compound-related changes included cortical (6/8) and medullary necrosis (4/8) and cortical atrophy of the thymus and hepatocyte hypertrophy (4/8), eosinophilic cytoplasmic changes (1/8), hydropic degeneration (3/8), and sinusoidal collapse (1/8) in the liver. Kidney changes

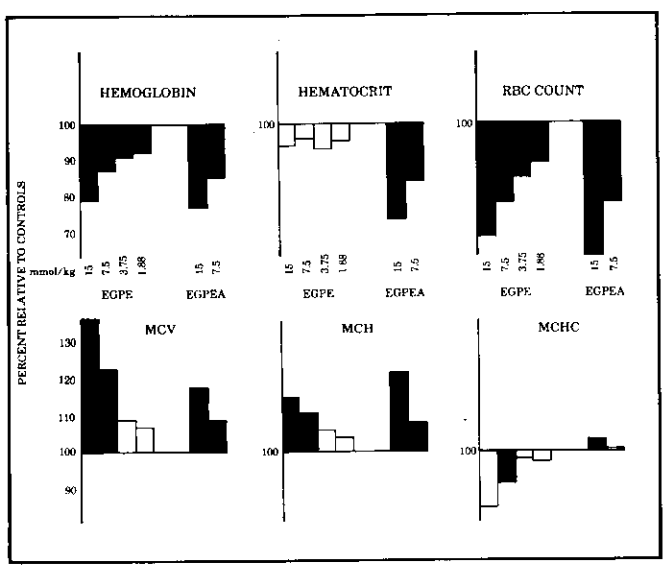

FIGURE 5. Comparative hematology of male rats exposed to EGPE and EGPEA (6-week gavage study). Bars indicate percent of control values: light bars, normal variation; dark bars, significantly different from controls ($p \leq 0.05$).

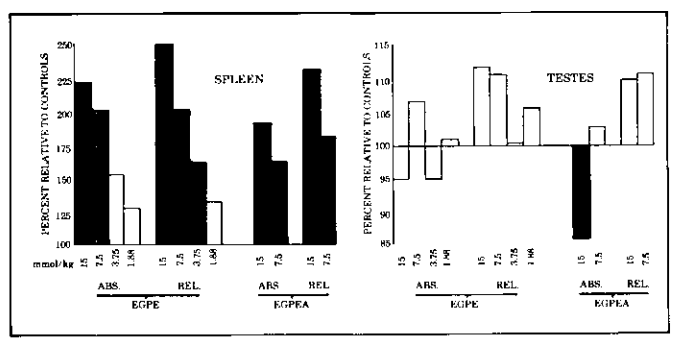

FIGURE 6. Comparative absolute and relative spleen and testes weights of rats exposed to EGPE and EGPEA (6-week gavage study). Bars indicate percent of control values: light bars, normal variation; dark bars, significantly different from controls ($p \leq 0.05$).

consisted of hyalin droplet degeneration (1/8) in the ascending limb of Henle's loop. Glomerular effusions (5/8) were also present. Splenic changes included lymphoid hypoplasia (2/8) and red pulp atrophy (5/8), while karyopyknosis (4/8) was seen in the mesenteric lymph nodes.

Gross pathologic findings for the remaining groups exposed to EGPEA and the groups exposed to EGPE are compared in Table 5. Splenic enlargement was seen in rats exposed to 15, 7.5 and 3.75 mmole/kg EGPE and 15 and 7.5 mmole/kg EGPEA. Testicular atrophy was seen in one rat and minimal hemorrhage in the nonglandular portion of the stomach in another rat exposed to 15 mmole/kg EGPEA.

Histologic lesions are compared in Table 6. EGPE produced congestion and extramedullary hematopoiesis of the spleen in animals exposed to 15, 7.5 and 3.75 mmole/kg. Renal effects included proteinaceous casts (15 mmole/kg only) and hemosiderin in the proximal convoluted tubules at all dose levels. Liver effects were focal hemosiderin in the 15 mmole/kg dose group.

Testicular changes seen in the 15 mmole/kg EGPEA group included cytoplasmic vacuolation in the seminiferous tubules and degenerated sperm in the lumen of the epididymides of one animal, while another rat had atrophy of the seminiferous tubules. A minimal to moderate incidence of brown pigment was seen in the proximal convoluted tubules of 9/10 rats. The only possible treatment-related change seen in the low dose group (7.5 mmole/kg EGPEA) was brown pigment in the convoluted tubules of eight of ten of the animals.

Two-Week Inhalation Studies. Mean analytic concentrations and standard deviations were 786 ± 91, 406 ± 24, 206 ± 13, 103 ± 10 and 0.0 ppm for EGPE and 825 ± 34, 389 ± 14, 203 ± 5, 92 ± 6 and 0.0 ppm for EGPEA. These compared favorably with target concentrations of 800, 400, 200, and 100 ppm.

Body weight changes are shown in Figures 7 and 8 for animals exposed to EGPE and Figures 9 and 10 for animals exposed to EGPEA. Body weight gains were comparable to control values for all groups of both compounds. A weight loss, seen on day 10 in the 100 ppm EGPE group, resulted from a malfunction in an automatic water system.

Hemoglobinuria (Table 7) confirmed by Ames N-Multistix-C reagent strips was observed on dropping trays beneath 2/5 males and 3/5 females exposed to 800 ppm EGPE and 1/5 males exposed to 400 ppm EGPE. Hemoglobinuria was observed in 4/5 males and 5/5 females exposed to 800 ppm EGPEA, 4/5 females exposed to 400 ppm EGPEA and 1/5 females exposed to 200 ppm EGPEA. Urine discoloration resolved in all animals after 2 days and did not reappear following subsequent exposures.

Hemoglobin concentration, hematocrit, red blood cell counts, and red blood cell indices (mean corpuscular volume, MCV; mean corpuscular hemoglobin, MCH; mean corpuscular hemoglobin concentration, MCHC) following exposure to EGPE and EGPEA are compared in Figures 11 and 12. In males, except for a slight but significant decrease in red blood cell counts following exposure to 800 ppm EGPE and EGPEA hemoglobin concentration, hematocrit and red blood cell counts were normal. Slight but significant increases were observed in MCV at 800 and 400 ppm EGPE and EGPEA, and in MCH at 800 and 400 ppm EGPE and 800 ppm EGPEA. MCHC values were comparable to controls.

Table 5. Comparative gross pathology following exposure to EGPE and EGPEA: 6-week gavage study.

Compound	Site	Lesion	15 mmole/kg	7.5 mmole/kg	3.75 mmole/kg	1.88 mmole/kg
EGPE	Spleen	Enlarged, dark	4[a]	5	1	0
EGPEA	Spleen	Enlarged	7	3	—[b]	—
	Testes	Atrophy	1	0	—	—

[a]Number of male rats responding with lesion: $N = 10$.
[b]Study not conducted at these doses.

Table 6. Comparative histopathology following exposure to EGPE and EGPEA: 6-week gavage study.

Compound	Site	Lesion[a]	15 mmole/kg	7.5 mmole/kg	3.75 mmole/kg	1.88 mmole/kg
EGPE	Spleen	Congestion	6[b]	5	10	0
		Extramedullary hematopoiesis	0	4	3	0
	Liver	Focal hemosiderin	3	0	0	0
	Kidney	Proteinaceous casts	8	0	0	0
		Hemosiderin	8	8	2	2
EGPEA	Spleen	Congestion	2	0	—[c]	—
	Kidney	Brown pigment	9	8	—	—
	Testes	Atrophy or cytoplasmic vacuolization of seminiferous tubules	2	0	—	—
	Epididymides	Degenerated sperm in lumen	1	0	—	—

[a]None of these lesions were seen in control animals.
[b]Number of male rats responding with lesions: $N = 10$.
[c]Study not conducted at these doses.

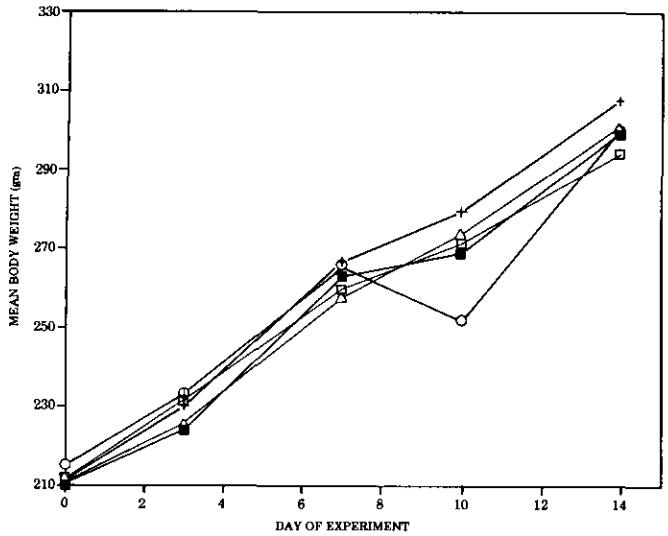

FIGURE 7. Mean body weights of male rats exposed to EGPE (2-week inhalation study): (■) 800; (+) 400; (Δ) 200; (○) 100 ppm; (□) controls.

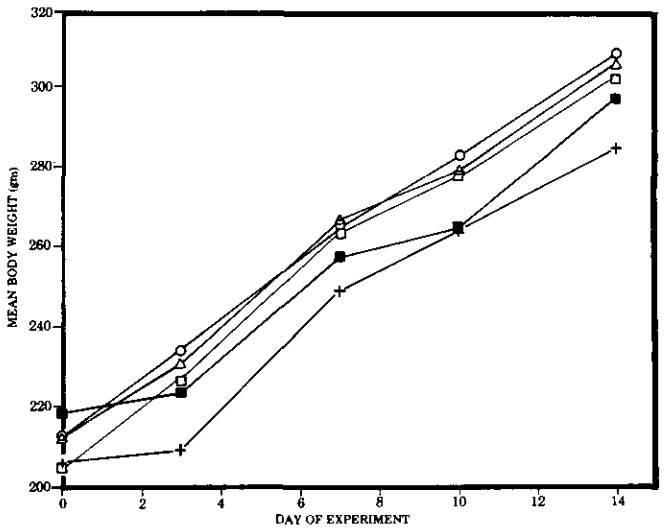

FIGURE 9. Mean body weights of male rats exposed to EGPEA (2-week inhalation study): (■) 800; (+) 400; (Δ) 200; (○) 100 ppm; (□) controls.

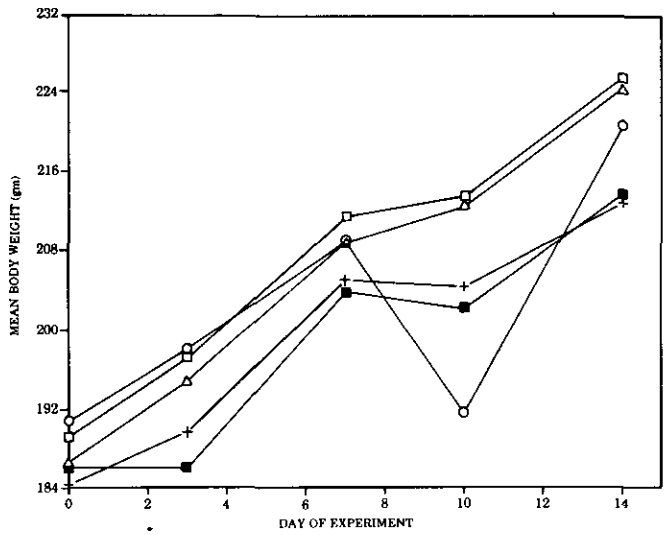

FIGURE 8. Mean body weights of female rats exposed to EGPE (2-week inhalation study): (■) 800; (+) 400; (Δ) 200; (○) 100 ppm; (□) controls.

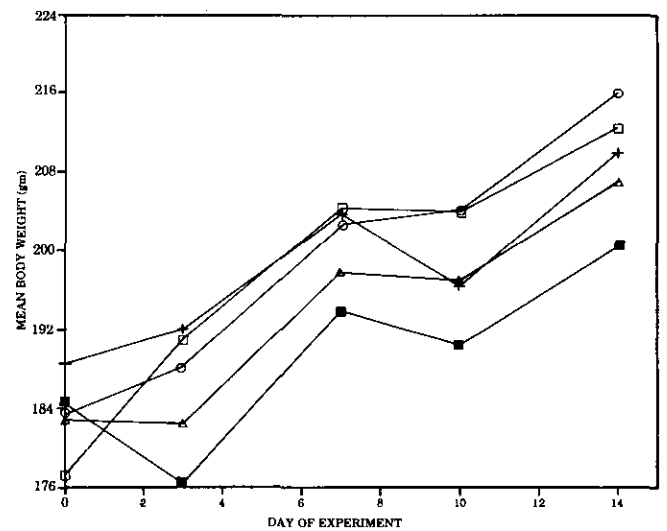

FIGURE 10. Mean body weights of female rats exposed to EGPEA (2-week inhalation study): (■) 800; (+) 400; (Δ) 200; (○) 100 ppm; (□) controls.

Only the red blood cell count was slightly but significantly decreased in females exposed to 800 ppm EGPE, while hemoglobin concentration, hematocrit, and red blood cell counts were decreased slightly in females exposed to 800 and 400 ppm EGPEA. MCV and MCH were significantly increased in females exposed to 800 and 400 ppm EGPE and 800 ppm EGPEA. MCHC was comparable to controls.

Reticulocytes were increased in both sexes after exposure to 400 ppm EGPE. Absolute and relative white blood cell counts and platelet counts were comparable to control values at all exposure levels for both compounds. Changes in red blood cell morphology observed at 800 and 400 ppm EGPE and EGPEA included increased polychromasia, anisocytosis, macrocytosis, microcytosis, stomatocytes, hypochromasia and Howell-Jolly Bodies. The number of animals showing these changes, as well as the severity of changes, were concentration dependent and considered significant at 800 and 400 ppm for both sexes and both compounds. No hematological signs of toxicity were seen in males and females exposed to 200 or 100 ppm EGPE and EGPEA.

Clinical chemistries (aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, urea nitrogen, creatinine, and sorbital dehydrogenase) were

Table 7. Hemoglobinuria following exposure to EGPE and EGPEA: 2-week inhalation study.[a]

	800 ppm		400 ppm		200 ppm		100 ppm	
	5M[b]	5F[b]	5M	5F	5M	5F	5M	5F
EGPE	2	2	1	0	0	0	0	0
EGPEA	4	5	0	4	0	1	0	0

[a]Observed on dropping trays. All signs resolved after second-third exposure.
[b]Number of rats examined.

normal in both sexes at all concentrations in animals exposed to EGPE. An increase in lactate dehydrogenase (100 ppm) and a decrease in glucose (100 and 800 ppm) in females were considered spurious and not toxicologically significant.

Clinical chemistry changes were seen in males only exposed to EGPEA and included a slight but significant decrease in serum urea nitrogen and alanine aminotransferase at 800 ppm and alanine aminotransferase at 200 and 100 ppm. None of these changes were of a magnitude to represent an untoward biologic effect and may represent spurious excursions frequently seen in the rat. All other clinical chemistries (aspartate aminotransferase, lactic dehydrogenase, alkaline phosphatase, glucose and creatinine) were normal.

Terminal body weights of all animals exposed to either compound were comparable to control values. Absolute and relative (to body weight) spleen weights (Fig. 13) were significantly increased in males and females exposed to 800 ppm EGPE and EGPEA and in males only at 400 ppm EGPEA. Relative spleen weights were also increased in males and females exposed to 400 ppm EGPE and EGPEA. No significant differences were seen in spleen weights of animals exposed to 200 and 100 ppm of either compound.

Absolute and relative (to body weight) liver, kidney, brain, heart and gonad weights of both sexes were normal at all exposure levels of EGPE.

Absolute and relative liver weights were increased slightly in females exposed to 800 and 100 ppm EGPEA

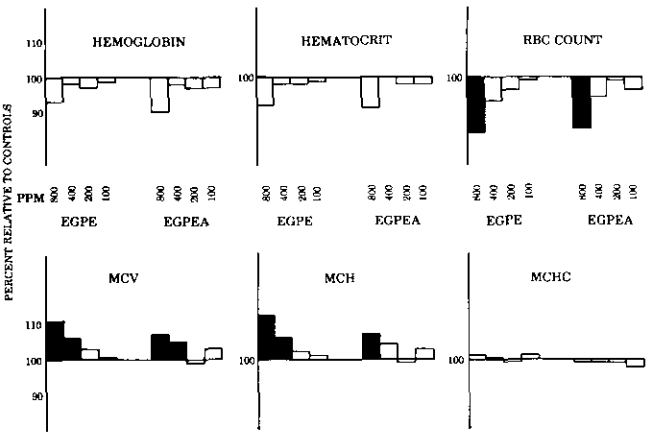

FIGURE 11. Comparative hematology of male rats exposed to EGPE and EGPEA (2-week inhalation study). Bars indicate percent of control values: light bars, normal variation; dark bars, significantly different from controls ($p \leq 0.05$).

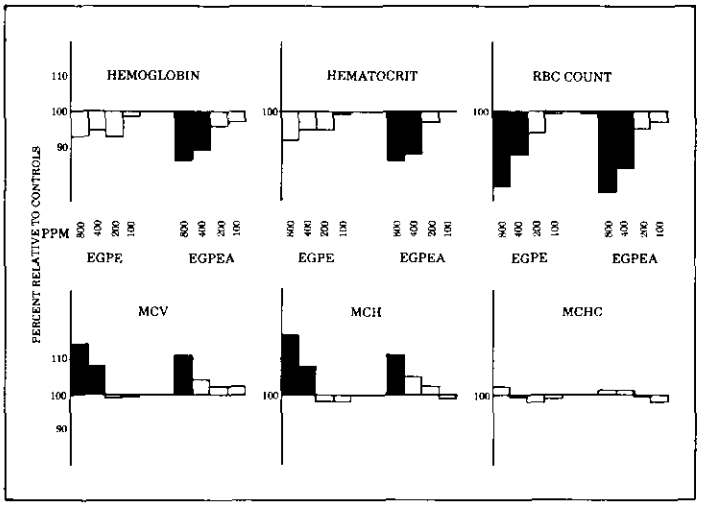

FIGURE 12. Comparative hematology of female rats exposed to EGPE and EGPEA (2-week inhalation study): bars indicate percent of control values; light bars: normal variation; dark bars: significantly different from controls ($p \leq 0.05$).

Table 8. Comparative gross and histopathology following exposure of rats to EGPE and EGPEA: 2-week inhalation study.

	800 ppm		400 ppm		200 ppm		100 ppm	
	5M[a]	5F[a]	5M	5F	5M	5F	5M	5F
EPGE								
Gross examination								
Dark spleens	2	0	0	0	0	0	0	0
Histologic examination								
Spleen congestion	3	4	2	2	0	0	0	0
Lymphoid hyperplasia	1	1	1	1	0	0	0	0
Extramedullary hematopoiesis	2	0	1	0	0	0	0	0
Hemosiderin	2	2	0	2	0	0	0	0
EGPEA								
Gross examination								
Abnormalities	0	0	0	0	0	0	0	0
Histologic examination								
Spleen: extramedullary hematopoiesis	5	5	0	0	0	0	0	0
Kidney: extramedullary hematopoiesis	0	5	0	0	0	0	0	0
Liver: hemosiderin	0	4	0	0	0	0	0	0

[a]Number of rats examined.

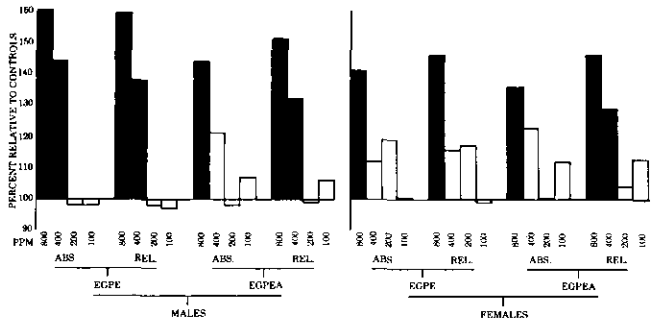

FIGURE 13. Comparative absolute and relative spleen weights of male and female rats exposed to EGPE and EGPEA (2-week inhalation study). Bars indicate percent of control values: light bars, normal variation; dark bars, significantly different from controls ($p \leq 0.05$).

and a slight increase in relative liver weight was seen in females exposed to 400 ppm. The increases were very slight and not considered biologically significant. Absolute and relative liver weights of males at all exposure concentrations were comparable to control values. All other absolute and relative organ weights (kidneys, brain, heart, gonads) were normal at all exposure concentrations in both sexes.

On gross examination (Table 8), the spleens of two of five male rats exposed to 800 ppm EGPE were dark and granular. No compound-related gross pathologic changes were observed in any of the other rats exposed to EGPE or in animals of either sex exposed to EGPEA.

Compound-related histopathologic changes (Table 8) seen in the spleens of both sexes exposed to 800 or 400 ppm EGPE included minor splenic congestion, lymphoid hyperplasia, extramedullary hematopoiesis, and hemosiderin. No other compound-related effects were observed in these animals or in either males or females exposed to 200 or 100 ppm of EGPE.

Histologic changes were seen in males and females exposed to 800 ppm EGPEA only. Males (5/5) had minor to moderate extramedullary hematopoiesis of the spleen. Females had minor pigmentation in the proximal convoluted tubules (5/5) of the kidneys, minimal to minor extramedullary hematopoiesis (5/5) of the spleen, and minimal to moderate hemosiderosis of the Kupffer cells (4/5) in the liver.

Discussion

EGPE and EGPEA exhibit a relatively low degree of acute toxicity following exposure by oral, inhalation, dermal and ocular administration. The primary site of systemic toxicity in rats appears to be the red blood cell with clinical evidence of hemoglobinuria following single doses of high concentrations orally and by inhalation. These findings are comparable to those of single-dose studies reported in the literature for EGPE (*1,11*). Bloody urine is also associated with exposure to the lower molecular weight derivatives of glycol ethers such as EGME, EGEE and EGEEA (*1*). EGPEA appears slightly less toxic acutely than EGPE.

Repeated exposures to EGPE or EGPEA either orally or by inhalation appear to produce similar responses in rats. The primary site of toxic action is the red blood cell with secondary effects seen in the liver, spleen and kidneys.

Orally, EGPEA (15 mmole/kg/day) produced testicular injury in two rats. No testicular changes were seen following oral administration of an equimolar dose of EGPE. Testicular injury has been reported for the lower molecular weight ethylene glycol derivatives EGME and EGEE and their esters, EGMEA and EGEEA, when administered by gavage (62.5–4000 mg/kg) to mice over a 5-week period (*12*). Leukopenia, which was also seen in this study (12) was not seen in the present study with EGPE and EGPEA.

Testicular injury has been reported also following repeated inhalation exposures to EGME in rats (1000 and 300 ppm, 6 hr/day) over a 2-week period (*13*). No testicular injury was seen following 2-week vapor exposures with as high as 800 ppm EGPE and EGPEA. Hematologic changes associated with the red blood cell population following EGPE and EGPEA vapor exposure are similar to those described in the literature following vapor exposure to EGME, EGMEA, EGEE and EGEEA. White blood cell and bone marrow changes were not observed in the present study.

Table 9 presents the no-observed effect levels (NOEL) for the various parameters studied in animals exposed to EGPE or EGPEA by the oral and inhalation routes. In general, when EGPE and EGPEA have toxicologic effects similar to those described in the literature for

Table 9. Comparative "no-observed effect level" (NOEL) following oral and inhalation exposure to EGPE and EGPEA.

	EGPE		EGPEA	
	Gavage, mmole/kg	Inhalation, ppm	Gavage, mmole/kg	Inhalation, ppm
Body weight	7.5	800	7.5	800
Clinical signs	< 1.88	200	< 7.5	100
Hematology	< 1.88	200	< 7.5	200
Clinical chemistry	15	800	15	800
Gross examination	< 1.88	200	< 7.5	200
Microscopic examination	< 1.88	200	< 7.5	400
Overall NOEL	< 1.88	200	< 7.5	100

EGME, EGMEA, EGEE, and EGEEA, on a relative basis (equimolar) EGPE and EGPEA are less potent than their lower molecular weight homologs.

Conclusion

EGPE and EGPEA exhibit a relatively low degree of acute toxicity following exposure of animals by oral, inhalation, dermal and ocular administration. The primary site of systemic toxicity after single-dose oral or inhalation exposure is the red blood cell.

The primary site of toxicity following oral exposure for 6 weeks or inhalation exposure for 2 weeks is the red blood cell with secondary changes produced in the liver, spleen and kidneys. Extremely high oral doses of EGPEA, but not an equimolar dose of EGPE, causes testicular injury. Inhalation exposure to concentrations as high as 800 ppm EGPE or EGPEA for 2 weeks does not produce testicular damage.

Although there are striking similarities between the toxicity of EGPE and EGPEA, they are less toxic on a relative basis (equimolar) than the lower molecular weight ethylene glycol derivatives EGME, EGMEA, EGEE and EGEEA.

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